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Random amplified polymorphic DNA variation among remnant big bluestem (*Andropogon gerardii* Vitman) populations from Arkansas' Grand Prairie

D. J. GUSTAFSON, D. J. GIBSON and D. L. NICKRENT
Department of Plant Biology, Southern Illinois University, Carbondale, IL 62901-6509, USA

Abstract

Random amplified polymorphic DNA (RAPD) analysis was used to characterize genetic diversity and genetic distinctiveness of *Andropogon gerardii* from remnant Arkansas prairies. Six oligonucleotide primers, which generated 37 RAPD bands, were used to analyse 30–32 plants from six Grand Prairie populations, Baker Prairie (Arkansas Ozarks), two Illinois prairies and two cultivars. Genetic diversity of the Arkansas remnants ranged from 82.7 to 99.3%, with 89% of the total genetic variation within and 11% among populations. The partitioning of genetic variation was consistent with that reported for other outcrossing perennial grasses, using the more conservative allozyme markers. Principal component analysis indicated a northern and southern association within Arkansas' Grand Prairie. Although there was no genetic structuring at the landscape level, the Illinois prairies and cultivars were different from all Arkansas prairies tested. There was significant within-population structuring in four of the seven Arkansas remnants, with a negative relationship between genetic similarity and geographical distance. The three nonstructured populations were from a linear railroad remnant, suggesting different population-level dynamics from nonlinear prairies. The results of this study indicated that small isolated remnant big bluestem populations were not genetically depauperate and that genetic relationships among populations could not be predicted solely on geographical proximity.

Keywords: *Andropogon*, cultivars, genetic diversity, prairie, RAPD

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Introduction

Big bluestem (*Andropogon gerardii* Vitman) is a native, polyploid, self-incompatible, warm-season, perennial prairie grass that is a widespread component of the North American prairie biome and is a dominant species of the tallgrass prairie (Weaver & Fitzpatric 1934; Keeler 1990; McKone *et al*. 1998). Fragmentation and habitat reduction of the North American prairie has coincided with the urbanization and agricultural development of the central United States (Risser 1988). The resulting small populations are more prone to extinction than the original larger populations because they are less resistant to environmental stochasticity, demographic variation and the reduction in genetic variability (Gilpin & Soule 1986). Species that naturally occur in small populations may posses genetic systems, such as selfing or mixed mating, which are adapted for close inbreeding. However, widespread and abundant outcrossing species, such as big bluestem, which have experienced dramatic reductions in population numbers, may be particularly susceptible to environmental and genetic stresses imposed by small population size (Barrett & Kohn 1991).

Genetic differentiation in grasses has been shown over a range of spatial scales, from metres (Hamrick & Allard 1972; Nevo *et al*. 1986) to large climatic regions (Rice & Mack 1991). Some studies of grasses have demonstrated an association between geographical distance and genetic similarity (Nevo & Beiles 1989; Castagne *et al*. 1997; Demissie *et al*. 1998), while others have shown no such relationship (Knapp & Rice 1996; Huff *et al*. 1998). Commercial sources of native grass seed are increasingly available for revegetation and restoration projects (Knapp & Rice 1996). The

Correspondence: D. J. Gustafson. Fax: +618-453-3441; E-mail: dgustaf@siu.edu

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use of commercial seed over large geographical areas has caused concern about local adaptation and maintenance of genetic integrity of existing native grass populations (Millar & Libby 1989; Linhart 1995). Therefore, molecular studies of genetic variation will provide the information required to assess and manage genetic resources more effectively.

The Grand Prairie region of eastern Arkansas, a component of the North American tallgrass prairie, consisted of 3.9×10^5 ha of continuous prairie prior to European settlement. Extensive conversion to agriculture at the turn of the 20th century has reduced the Grand Prairie to 11 isolated remnant prairies constituting 227 ha (Irving *et al*. 1980). This dramatic reduction in population size and increased spatial isolation should result in decreased genetic diversity and increased population divergence, presumably owing to genetic drift, and reduced gene flow (Young *et al*. 1996).

The purpose of this study was to use random amplified polymorphic DNA (RAPD) to investigate genetic relationships of a dominant native grass, big bluestem, from remnant prairies in Arkansas' Grand Prairie, remnant populations distinct from and outside the Grand Prairie, and two commercially available cultivars ('Roundtree' and 'Kaw'). RAPD markers have proven to be a powerful tool for assessing population structure of grasses (Dawson *et al*. 1993; Huff *et al*. 1993; Gunter *et al*. 1996; Castagna *et al*. 1997; Huff *et al*. 1998), are not affected by variation in ploidy levels (Weising *et al*. 1995) and can detect genotypic variation in populations determined to be monomorphic using allozymes (Waycott 1998). The specific objectives were threefold. First, to characterize genetic distinctiveness and genetic diversity. Second, to determine genetic structure. Finally, to assess whether there was a correlation between vegetation estimates (percentage cover of big bluestem, other grasses, forbs, total cover), size of the remnant and genetic diversity of remnant big bluestem populations from the Grand Prairie of Arkansas.

Materials and methods

Study sites

Four remnant prairies from the Grand Prairie region of Arkansas and Baker Prairie (from the Arkansas Ozarks) were sampled in this study (Fig. 1). The largest remaining remnant prairie was the Railroad Prairie, which was 21 km long and between only 32 and 54 m wide (T. Foti, personal communication). In the event that the shape of the prairie would influence population dynamics, we collected from three different locations within the Railroad Prairie. Samples were collected from the west and middle areas, which are located at opposite ends of a contiguous 16.2 km section, and from the 4.8 km east area, which is separated from the middle area by the town of Hazen, Arkansas. Overall, the Arkansas remnant prairies ranged in size from 11.8 to 32.7 ha and encompassed ≈ 76% of the remaining Grand Prairie remnants. The time since prairie reduction and management practices were approximately the same for the Arkansas remnants sampled (T. Foti, personal communication). To estimate the distinctiveness of Arkansas prairies, DeSoto Railroad Prairie (Jackson County, Illinois) and Weston Cemetery Prairie (McLean County, Illinois) were included in the study. DeSoto Railroad

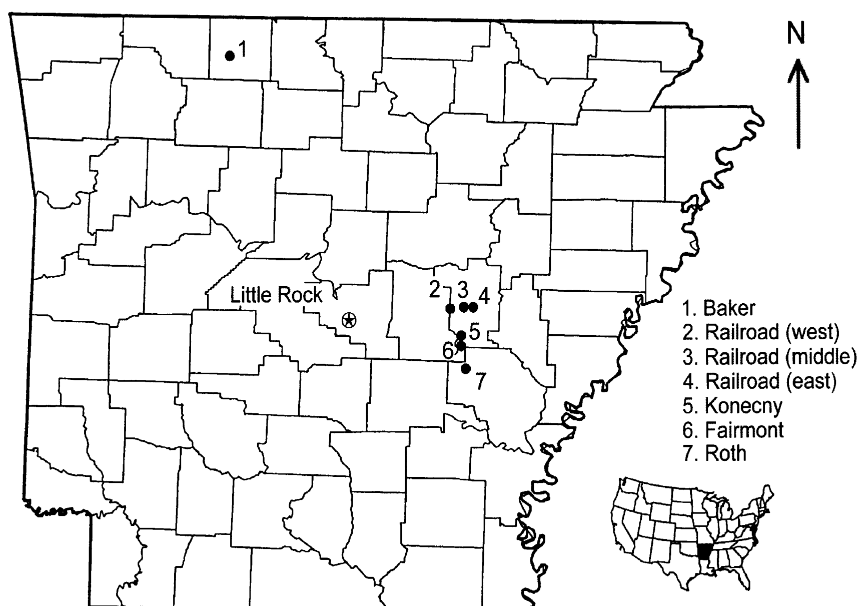

Fig. 1 Arkansas map showing the seven remnant tallgrass prairies used in this study.

Prairie and Weston Cemetery Prairie were located ≈ 440 and 750 km northeast of the Arkansas Grand Prairie region, respectively. Two commercially available cultivars ('Roundtree' and 'Kaw') were included in this study. The original seed source for 'Roundtree' was from Moorhead (Iowa, USA), and was developed and released from the Plant Materials Center, Elsberry (Missouri, USA) in 1983 (HTTP://Plant-Materials.NRCS.USDA.GOV). The 'Kaw' cultivar source was originally from Riley County (Kansas, USA), developed and released from the Plant Materials Center, Manhattan (Kansas, USA) in 1950 (HTTP://Plant-Materials.NRCS.USDA.GOV).

Population sampling

Between 30 and 32 flowering plants were randomly sampled along two parallel transects (at least 20 m apart), at each of the seven Arkansas prairies, using a randomly stratified sampling procedure. Plants were sampled at least 10 m apart, the spacing being chosen to minimize the chance that each collection was taken from the same clone. Rhizome and leaf material from individual big bluestem plants were collected (on 13–14 August 1996), stored on ice and transported to the Southern Illinois University at Carbondale (SIUC). Seeds of big bluestem from Weston Cemetery Prairie and DeSoto Railroad Prairie were collected during October 1995. Thirty plants were randomly sampled along a transect, maintaining at least 10 m between consecutive samples, that traversed the remnant. Bulk samples containing seeds from 100 to 300 additional plants were collected at the Weston and DeSoto sites and used to augment the number of individuals in the event that individual collections had not produced adequate seed. These seeds were cold stratified for 3 months, germinated and grown to seedling stage in a greenhouse. 'Roundtree' and 'Kaw' seed (Sharp Brothers Seed) from the 1995 season were also grown in a greenhouse. At each site, the percentage cover was estimated for big bluestem, other grasses, forbs and total vegetation using 10 randomly stratified 10 m^2 circular quadrats and the modified Daubenmire cover class scale (Abrams & Hulbert 1987).

DNA extraction

Genomic DNA was extracted from 3-month-old seedlings and the vegetative material collected in Arkansas. Fresh leaf tissue (1.0 g) was ground to a powder in liquid nitrogen and 1.3 mL of 94 °C CTAB buffer: 100 mM Tris-HCl (pH 8.0), 1.4 M NaCl, 30 mM EDTA, 2% (w/v) cetyltrimethylammonium bromide (CTAB) was added, with continued grinding. Three units of proteinase K and 60 µL of dithiothreitol (0.5 M) were added, mixed, transferred to 2.0-mL microfuge tubes and incubated for 60 min at 37 °C. The samples were extracted with chloroform : isoamyl alcohol (24 : 1) and centrifuged at 12 000 *g* for 15 min at 4 °C. The aqueous layer was transferred to a 1.5-mL microfuge tube and 0.5 mL of –20 °C isopropanol was added, mixed and the sample stored in a –20 °C freezer for 30 min. The DNA was pelleted, air dried and resuspended in 300 µL of TE buffer (1 mM Tris-HCl, 0.1 mM EDTA pH 8.0). Two-hundred microlitres of 4 M ammonium acetate and 1.0 mL of –20 °C (100%) ethanol were added, and the sample was stored at –20 °C for 60 min. The DNA was then pelleted, air dried and dissolved in 100 µL of TE buffer with RNase (1 U). The DNA concentration of each sample was quantified by comparison with a standard of known concentration on agarose gels stained with ethidium bromide. The genomic DNA was diluted to a working concentration of ≈ 5 ng/µL.

RAPD profiling

Thirty 10-base oligonucleotide primers (Operon Technologies) were surveyed and six were selected that showed intense and reproducible bands. The following primers (with sequence and number of bands scored in parentheses) were used: A-17 (dGACCGCTTGT, six), B-01 (dGTTTCGCTCC, seven), B-04 (dGGACTGGAGT, five), B-07 (dGGTGACGCAG, six), B-12 (dCCTTGACGCA, seven) and N-13 (dAGCGTCACTC, six). Amplification reactions were performed in 25-µL volumes containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton-X-100, 1.2 mM $MgCl_2$, 0.2 mM of each dNTP (Pharmacia), 20 ng of primer, 0.2 µL of *Taq* polymerase (Promega) and ≈ 5 ng of template DNA. One negative control, containing all of the amplification reagents and no template DNA, was run with each reaction. Samples were overlaid with 30 µL of mineral oil. Amplifications were performed in a Stratagene Robocycler (model no. 400 860; Stratagene) programmed with the following parameters: 94 °C for 3 min, followed by 45 cycles of 94 °C for 1 min, 36 °C for 1 min, 72 °C for 2 min, and a final incubation at 72 °C for 10 min. Amplification products (5 µL) were electrophoresed in 1.0% TBE (0.089 M Tris-HCl, 0.089 M boric acid, 0.002 M EDTA pH 8.0) agarose gels. After the dye had migrated for 15 cm, gels were stained with ethidium bromide, scanned and stored as a digitized image file with the Gel-doc 1000 system (Bio-Rad Laboratories). The Molecular Analyst (Bio-Rad Laboratories) software package was used to adjust the contrast, thus enhancing the visualization of the DNA bands. A DNA molecular weight marker (Promega; no. G1741) was used to standardize band intensities and to estimate the size of the RAPD bands. If cross-contamination was indicated by the presence of amplification products in the negative control lane, the run was repeated.

Generally, these primers produced complex banding profiles; however, only five to seven reproducible bands, between 0.7 and 2.6 kb, were scored for each primer.

Because the number of bands scored per primer was essentially the same (five to seven), the likelihood of a single primer biasing the analysis was low. The presence or absence of 37 bands constituted the genotype score for each sample.

Data analysis

Relationships among populations were investigated using principal component analysis (PCA) and parallel analysis (PA) to establish which PCA axes were appropriate for interpretation (SAS 1989). PA, for decomposing a correlation matrix, was used to derive the 95th percentile eigenvalues for each component, based on Monte Carlo analysis of the Longman *et al.* (1989) regression equations. Only axes with eigenvalues greater than the PA eigenvalues were significant ($\alpha = 0.05$) and retained for interpretation (Franklin *et al.* 1995).

Diversity in the frequency of RAPD band profiles per primer was apportioned within and among populations using Shannon's (Shannon & Weaver 1949) information measure:

$$H' = -\Sigma p_i \log_2 p_i$$

where p_i is the frequency of the band (when present). This measure is appropriate for qualitative data and can be separated into nested hierarchical levels. H' was calculated for two levels of RAPD band frequency: H'_{pop} was the average diversity within populations and $H'_{sp.}$ was the diversity of the species. The proportion of diversity within populations was the average (H'_{pop}) for all populations divided by species diversity ($H'_{sp.}$). The proportion of diversity among populations was the total diversity minus the average diversity among all populations, divided by the species diversity (King & Schaal 1989).

Genetic structure was investigated at broad (landscape) and narrow (population) geographical distances. Mantel's test was used to determine associations between Roger's genetic distance and geographical distance between populations using the computer program NTSYS-PC version 1.8 (1993). Within-population structuring using the Jaccard (1908) similarity coefficient and geographical distance between individual plants was also tested with Mantel's test (NTSYS-PC 1993). The Jaccard similarity coefficient is:

$$S_j = a/(n - \mathrm{d})$$

where a is the number of positive matches (1,1) n is the total sample size and d is the number of negative matches (0,0). The coefficient of Jaccard does not score negative matches as informative (Legrendre & Legrendre 1983).

Vegetation data among the Arkansas prairies were analysed using a one-way analysis of variance (general linear model with prairie as the dependent treatment) with the Tukey (honest significant difference) means separation procedure (SAS 1989). Spearmans' rank correlation was used to investigate associations between vegetation estimates, size of the remnant and genetic diversity (SAS 1989).

Results

Thirty-seven bands were used to evaluate genetic diversity and genetic relationships among the seven remnant Arkansas populations (Appendix I). PCA was used to investigate relationships among the six Grand Prairie populations, Baker Prairie, two Illinois prairies and two cultivars (Fig. 2). The first three axes of the PCA explained 60% of the cumulative variance. The first axis accounted for 28% of the variance and shows the separation of the Arkansas and non-Arkansas populations. The second (18%) and third (14%) delineated the genetic relationships within Arkansas, with a southern association comprising Roth and Fairmont Prairies and a northern association of Konecny and the three Railroad Prairies. Baker Prairie, from the Arkansas Ozarks, was associated with the northern Grand Prairie group. Illinois' Weston Cemetery Prairie and DeSoto Railroad Prairie formed a group, while the two cultivars grouped together. The Illinois prairies and cultivars were not clustered with the Arkansas Prairie.

Genetic diversity ranged from 82.7 to 99.3%, with 89% of the total genetic variation within and 11% among the remnant Arkansas populations (Table 1). Mantel's test was used to investigate genetic structure at landscape and population levels. At the landscape level, there was no

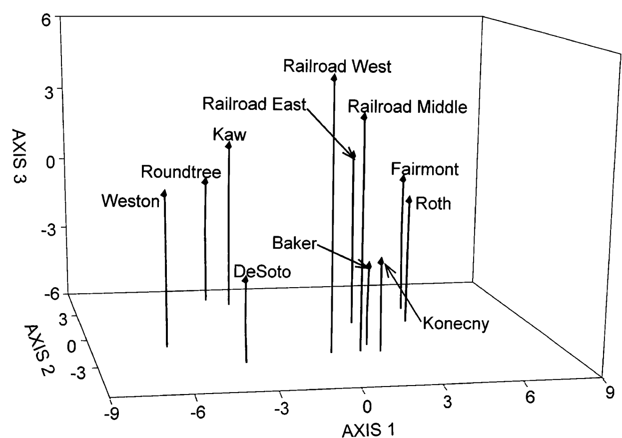

Fig. 2 Principal component analysis (PCA) depicting the relationships among six Arkansas Grand Prairie populations, one non-Grand Prairie Arkansas population (Baker), two Illinois populations (Weston and DeSoto) and two cultivars ('Roundtree' and 'Kaw') of big bluestem, based on the first three PCA axes.

Table 1 Genetic diversity and total area of the Arkansas prairies

Population	Area (ha)	Diversity (*H′*)
Railroad Prairie		
East	32.6	88.3
Middle	32.6	85.9
West	32.6	82.7
Konecny Prairie	11.7	99.3
Roth Prairie	16.2	88.8
Fairmont Prairie	12.1	88.7
Baker Prairie	28.7	94.2

significant association ($Z = -0.03$, $P = 0.4$) between Roger's genetic distance and geographical distance among the seven Arkansas sites. This nonsignificant result was a consequence of the association between the geographically separated Baker and Konecny Prairies. Significant within-population structuring was observed in all but the three Railroad Prairies (Table 2). The negative matrix correlation coefficients for the four structured populations ranged from −0.09 to −0.16, indicating a negative relationship between genetic similarity and geographical distance between individual plants.

Analysis of the vegetation data indicated that the Railroad East site had significantly higher total vegetation cover than the other sites, mainly as a result of a high cover value for non-big bluestem plants. Konecny and Roth Prairies had significantly higher forb cover than Fairmont and Baker Prairies. There was no significant difference in big bluestem cover among populations (Table 3). Percentage cover of big bluestem, other grasses, forbs and total vegetation were not correlated with genetic diversity. However, genetic diversity was negatively correlated ($r_s = -0.81$, d.f. = 6, $P = 0.01$) with size of the remnant prairie.

Discussion

Analysis of RAPD data of the Arkansas big bluestem populations revealed high levels of within-population genetic variability (83–99%) and low levels of population

Table 2 Results of the Mantel's tests between the Jaccard similarity coefficient and geographical distance between individual plants within each Arkansas population

Population	Matrix correlation (r) (= normalized Z statistic)	Approximate Mantel's *t*-test	Probability (random Z < observed Z)
Railroad Prairie			
East	−0.04	−0.56	0.28
Middle	−0.04	−0.61	0.26
West	0.01	0.16	0.56
Konecny Prairie	−0.15	−2.23	0.01*
Roth Prairie	−0.14	−2.59	0.01*
Fairmont Prairie	−0.09	−1.64	0.05*
Baker Prairie	−0.16	−2.37	0.01*

*Significant genetic structuring ($P < 0.05$).

Table 3 Percentage cover estimates (mean ± 1 SE) for big bluestem, other grasses, forbs and total vegetation cover from the Arkansas prairies

Population	Big bluestem	Other grasses	Forbs	Total cover
Railroad Prairie				
East	16.2 ± 5.0 a*	88.5 ± 8.8 a	15.9 ± 3.9 ab	121.1 ± 9.4 a
Middle	15.8 ± 7.3 a	44.1 ± 7.1 b	19.6 ± 5.6 ab	80.3 ± 4.6 b
West	15.7 ± 6.2 a	46.2 ± 6.7 b	16.7 ± 4.8 ab	79.1 ± 5.9 b
Konecny Prairie	20.4 ± 4.9 a	32.5 ± 7.3 b	31.0 ± 6.9 b	84.4 ± 7.4 b
Roth Prairie	13.8 ± 6.5 a	32.8 ± 5.8 b	36.0 ± 6.5 b	83.1 ± 9.9 b
Fairmont Prairie	7.1 ± 3.6 a	30.6 ± 4.3 b	6.6 ± 1.8 a	44.8 ± 2.7 c
Baker Prairie	28.2 ± 8.7 a	31.8 ± 8.8 b	10.9 ± 2.0 a	71.4 ± 4.8 b

*Values followed by different letters within a column are significantly ($P < 0.05$) different by the Tukey HSD multiple comparison test.

divergence (11%). These results were consistent with other RAPD analyses of outcrossing perennial grasses, with 5–15% of the genetic variation partitioned among populations (Huff *et al*. 1993, 1998; Huff 1997). Allozyme analysis of outcrossing species tends to have 17% of the total genetic variation residing among populations (Hamrick & Godt 1997), while outcrossing grasses exhibit 11% of the genetic variation among populations (Godt & Hamrick 1998). Although RAPD analysis has a greater potential for detecting DNA sequence variability than allozymes (Lui & Furnier 1993), the partitioning of genetic variation appears to be influenced more by the life history traits of the species than by the methodology.

Genetic diversity was negatively correlated with the size of the prairie remnant. This finding is not related to the time since reduction because these remnant prairies were isolated at approximately the same time. Management history was also very similar, with the prairies being mowed and burned infrequently (1900–75) and biannually (1976 to present). This negative correlation with size could be a consequence of incomplete sampling of the linear-shaped Railroad Prairie, different population dynamics in linear vs. nonlinear shape prairies, or selection favouring heterozygous individuals in the smaller remnants (Mitton 1989). Whether sampling error, shape of the remnant prairie or heterosis affects estimates of genetic diversity, it is clear that the small remnant big bluestem populations possess high levels of genetic diversity. Therefore, even small 'postage stamp'-size remnants contribute much to the overall genetic diversity of the species.

The high level of genetic variation may be because big bluestem is a cytotypically complex allopolyploid species, with hexaploid ($2n = 6x = 60$) and enneaploid ($2n = 9x = 90$) cytotypes. These cytotypes are self-incompatible, cross freely between cytotypes and produce fertile euploids and aneuploids (Norrmann *et al*. 1997). In addition, allopolyploid species typically arise from two or more independent origins, incorporate genetic variability (through hybridization of related populations) without reliance on mutation to introduce allelic variation and are characterized by fixed heterozygosity (Stebbins 1975; Lewis 1979; Soltis *et al*. 1992). Complex polyploid species, such as big bluestem, may be better 'reservoirs' of genetic diversity than diploid species and this may at least partially explain the high levels of genetic variation.

The Grand Prairie of Arkansas consisted of two genetic associations, with Fairmont and Roth Prairies constituting the southern group and Konecny and Railroad Prairie sites the northern group. If genetic relationships could be predicted by geographical proximity, then Baker Prairie (northern Arkansas) should be genetically distinct from all Grand Prairie populations. However, Konecny Prairie was genetically more similar to Baker Prairie than to the two prairies (Roth and Fairmont) located within 20 km. Therefore, designation of a 'local' genotype to be used for restoration projects should be determined empirically rather than by assuming that close proximity predicts genetic similarity.

The two cultivars, which were developed as forage crops, were genetically similar to one another and genetically different from the Illinois and Arkansas populations. Genetic similarity between the cultivars could be a consequence of selection for forage quality traits or the result of many generations of cultivation. Commercial sources of native and cultivar grass seed rely upon agricultural practices to increase seed production but, as shown with native populations of *Festuca pratensis*, intensive management practices can affect genetic variability (Kolliker *et al*. 1998).

An increased emphasis is being placed on empirical measures of genetic diversity to properly assess and manage genetic resources. Conservation managers and restoration ecologists recognize the importance of understanding the genetic consequences of their management decisions (Schaal & Leverich 1992). However, most genetic studies of threatened ecosystems focus on threatened or endangered species. In this study, we investigated the genetic relationships within and among populations of the dominant species. This approach should provide a better measure of the ecosystem's genetic health than results derived from the rare members of the community. The results of this study show that small isolated remnant big bluestem populations were not genetically depauperate, and that genetic relationships among populations could not be predicted solely on geographical proximity.

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The results presented in this paper will form part of Danny J. Gustafson's dissertation: *Genetic and Ecological Consequences of Habitat Reduction and Fragmentation of Remnant and Restored Tallgrass Prairies*. Daniel Nickrent's laboratory applies molecular methodologies to systematic and evolutionary questions, with an emphasis on parasitic flowering plants. David Gibson's laboratory studies all aspects of plant populations and community ecology with a focus on grassland dynamics.

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Appendix I

Band frequencies for each population

	East (30)	Middle (32)	West (32)	Konecny (32)	Roth (32)	Fairmont (32)	Baker (32)	DeSoto (32)	Weston (20)	Kaw (32)	Roundtree (32)
A-17											
1	0.400	0.250	0.063	0.406	0.344	0.438	0.500	0.455	0.450	0.350	0.500
2	0.500	0.250	0.188	0.813	1.000	0.938	0.938	0.303	0.450	0.419	0.688
3	0.333	0.438	0.125	0.375	0.250	0.406	0.375	0.212	0.100	0.258	0.344
4	0.300	0.000	0.000	0.375	0.250	0.344	0.156	0.000	0.000	0.548	0.563
5	0.600	0.375	0.625	0.531	0.656	0.469	0.688	0.788	0.450	0.452	0.438
6	0.800	0.938	0.938	0.813	0.844	0.813	0.781	0.636	0.450	0.581	0.531
B-01											
1	0.367	0.710	0.563	0.645	0.125	0.065	0.839	0.909	0.900	0.906	0.793
2	0.067	0.355	0.375	0.419	0.188	0.129	0.548	0.606	0.800	0.688	0.828
3	0.900	0.742	0.594	0.742	0.281	0.419	0.677	0.788	0.800	0.969	1.000
4	0.433	0.226	0.688	0.452	0.281	0.258	0.516	0.063	0.600	0.313	0.379
5	0.900	0.548	0.781	0.548	0.500	0.548	0.516	0.394	0.600	0.781	0.517
6	0.667	0.581	0.844	0.452	0.438	0.452	0.710	0.697	0.950	0.813	0.724
7	0.300	0.226	0.188	0.290	0.438	0.484	0.226	0.152	0.200	0.344	0.276
B-04											
1	0.367	0.250	0.000	0.531	0.125	0.063	0.531	0.219	0.100	0.281	0.000
2	0.567	0.031	0.156	0.313	0.438	0.250	0.375	0.375	0.050	0.156	0.406
3	0.067	0.563	0.313	0.281	0.906	0.719	0.313	0.813	0.500	0.875	0.656
4	0.800	0.688	0.781	0.688	0.938	0.813	0.938	0.531	0.900	0.969	1.000
5	0.500	0.750	0.625	0.281	0.844	0.906	0.344	0.313	0.600	0.781	0.719
B-07											
1	0.333	0.188	0.438	0.344	0.313	0.344	0.219	0.576	0.750	0.344	0.406
2	0.167	0.469	0.406	0.188	0.156	0.219	0.375	0.545	0.550	0.313	0.406
3	0.267	0.156	0.156	0.344	0.125	0.250	0.250	0.485	0.700	0.250	0.313
4	0.700	0.469	0.625	0.344	0.594	0.500	0.531	0.333	0.550	0.531	0.500
5	0.533	0.563	0.438	0.625	0.813	0.781	0.625	0.788	0.850	0.875	0.500
6	0.667	0.563	0.594	0.781	0.344	0.344	0.406	0.727	0.700	0.625	0.750
B-12											
1	0.103	0.125	0.563	0.250	0.125	0.125	0.188	0.273	0.350	0.387	0.621
2	0.207	0.438	0.438	0.406	0.469	0.156	0.375	0.515	0.450	0.226	0.241
3	0.759	0.719	0.781	0.719	0.813	0.781	0.719	0.788	0.750	0.806	0.931
4	0.276	0.625	0.406	0.406	0.281	0.156	0.313	0.485	0.600	0.548	0.414
5	0.517	0.344	0.438	0.344	0.531	0.344	0.469	0.697	0.800	0.677	0.655
6	0.724	0.469	0.594	0.688	0.500	0.688	0.688	0.727	0.750	0.710	0.897
7	0.172	0.000	0.000	0.344	0.344	0.219	0.531	0.152	0.000	0.065	0.138
N-13											
1	0.267	0.258	0.563	0.645	0.500	0.563	0.452	0.515	0.200	0.344	0.300
2	0.700	0.387	0.281	0.258	0.531	0.563	0.548	0.485	0.800	0.875	0.767
3	0.800	0.871	0.750	0.581	0.719	0.844	0.484	0.848	0.700	0.875	0.833
4	0.867	0.935	0.844	0.839	0.906	0.875	0.968	0.939	1.000	0.531	0.200
5	0.067	0.032	0.125	0.161	0.281	0.094	0.226	0.606	0.350	0.625	0.233
6	0.467	0.419	0.438	0.226	0.375	0.531	0.290	0.091	0.000	0.688	0.200

The sample size for each population is presented in parenthesis below each location.